Application of High Pressure Processing to Virginia's Oyster Industry

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SUMMARY

High pressure processing (HPP) has been shown to be an effective non-thermal processing treatment for shellfish. The consumption of raw oysters has been identified as a potentially serious food safety hazard for both normal and at-risk individuals. HPP can be used to reduce populations of *Vibrio* spp. in oysters while also releasing the abductor muscle from the shell. The Virginia oyster and quahog clam industries can benefit from the reduced labor needed to shuck the shellfish, an increased shelf life, and the reduced risk of selling a potentially hazardous product. Research indicates that *Vibrio* spp., fecal coliforms, and aerobic plate counts are all reduced through treatment with HPP.

Furthermore, the Virginia shellfish industry has responded favorably to the concept of HPP. The Virginia Tech High Pressure Processing Laboratory has been able to educate industry leaders on the concepts and benefits of HPP though conferences, trade shows, and demonstrations.

CHAPTER 1:

PURPOSE

To demonstrate the effect of high pressure processing (HPP) on facilitating the shucking process of oysters and quahog clams and determine the effect of the process on selected physical and chemical properties.

MATERIALS AND METHODS

Sample Preparation and Processing

Eastern oysters (*Crassostrea virginica*) harvested from the Gulf Coast were obtained from Cowart Seafood (Lottsburg, VA) and transported on ice to Virginia Tech (Blacksburg, VA). Oysters were collected in the fall of 2003 and again in the summer of 2004 to minimize the effects of seasonal differences. Oysters were placed in a metal basket and processed in a 35L high pressure processor (Quintus Press type QPF 35L-600; Avure Technologies, Kent, WA) with a water tank temperature of 75 °C. A hydrostatic pressure of 275 MPa was attained in 101 sec with an adiabatic temperature rise of 12 °C.

Pressurization time for 310 MPa was 106 s with a temperature increase of 14 °C. Decompression time was less than 1 s for all runs. Two replications of each treatment were preformed during each seasonal sampling. The time/temperature combinations used were as follows: (1) control, no pressure treatment; (2) 275 MPa for 30 s; (3) 275 MPa for 60 s; and (4) 310 MPa for 30 s. Oysters were shucked, placed in a blowing tank for 5 min and subsequently placed on a skimming table for 3 min. The oysters were then subsampled for shelf life studies and stored in plastic containers at 4 °C. Analysis was conducted 2, 5, 10, 15 and 20 d after HPP. Unshucked oysters were also held for 7 d under refrigerated storage prior to HPP and subsequent analyzed to examine effects on storage prior to HPP.

Microbiological Analysis

Fifty grams of oysters were aseptically transferred to a sterile filtered Stomacher bag with 50 g of phosphate buffered saline solution (PBS) and massaged by hand through the bag for 1 min. Twenty milliliters of the filtered sample was aseptically transferred to 80 ml of PBS to achieve a 1:10 dilution. Serial dilutions were then made using 9 ml PBS blanks which were used to inoculate Petrifilm aerobic count plates, three-tube most probable number series of alkaline peptone water (APC, 10 ml aliquots), and five-tube most probable number series of lauryl sulfate tryptose broth (LST, 10 ml aliquots). Petrifilm aerobic count plates were counted after 48 h of incubation at 35 °C.

After 24 h incubation at 35 °C, one loopful of the top inch of fluid of the APW was streaked onto thiosulfate citrate bile salts (TCBS) agar and onto modified cellobiose polymyxin colistin (mCPC) agar. Plates were incubated for 24 h at 35 and 39 °C, respectively. Yellow colonies on mCPC agar were subcultured onto long-term storage agar and confirmed for *Vibrio vulnificus* (Vv) using API 20E tests. Green colonies on TCBS agar onto long-term storage agar and tested for *Vibrio parahaemolyticus* (Vp) using API 20E test. Most probable number results for Vp and Vv were determined using both growth and conformation data.

After 24 h one loopful of LST was transferred to tubes of EC broth with inverted Durham tubes and incubated at 45 °C for 24 h. Most probable number for fecal coliforms was determined using the number of EC broth tubes with gas formation. Microbiological analysis was preformed in duplicate for each replication.

Physical Analysis

Driploss was determined by placing 5 pre-weighed oysters on a skimming table for 2 min and then weighing the drained oysters. Percent driploss was determined using the following equation:

$$\% \text{ Driploss} = [(\text{Original weight} - \text{Drained weight}) / \text{Original weight}] * 100$$

Color was measured using L*, a*, and b* values on a Minolta colorimeter.

Effects on shucking were determined by the amount the abductor released from the shell: fully, partially, or no release. Full release was identified as a slightly open shell with no muscle attachment to the shell. Some muscle attachment with a slightly open shell indicated partial release. No release was identified by an intact oyster with a fully closed shell and the abductor muscle fully attached to the shell.

Sensory Analysis

Differences in odor between fresh frozen oysters and treatments were determined by a triangle test using untrained panelists (n = 28) recruited from the Food Science and Technology Department at Virginia Tech. Panelists were presented with four trays each containing three oysters at room temperature under red lights. Oyster were individually placed in Petri dishes and coded with a three-digit randomly generated number. Panelists were asked to determine which sample of the three presented had the different odor.

Statistical Analysis

Analysis of variance procedures were used to evaluate the data using SAS (SAS Institute Inc., Cary, NC). Tukey's HSD was used to determine mean separation.

RESULTS AND DISCUSSION

Microbiological Analysis

The microbiological data from this study suggests that the use of high pressure processing does produce a decrease in the overall microbial population as well as the population of pathogenic bacteria. Initial aerobic plate counts were decreased by 1 to 2 logs with the use of high pressures when compared to the control (Figures 1 & 2). All treatments produced aerobic plate counts statically lower than the control. A treatment of 310 MPa for 30 s had lower initial counts than the treatment of 275 MPa for 30 s in both seasonal studies. Populations remained below the control until at least 10 d after processing. During the summer shelf life study, aerobic plate count remained lower in the treated samples over the 20 d period. Oysters harvested in the fall had lower aerobic plate counts for 10 d after processing. Similar initial aerobic counts were found in oyster harvested in both the fall and summer months with similar decreases achieved with HPP treatments.

High pressure processing also decreased fecal coliforms over the shelf life of the oysters (Figures 3 & 4). Higher initial fecal coliform counts were observed in the summer with a significant one-log (10 percent) decrease observed when a treatment of 310MPa for 30s was applied. Oysters harvested in the fall had lower initial fecal coliform counts with no significant difference between treatments and the control.

Vibrio vulnificus populations were reduced 3 logs (99.9 percent) by high pressure processing, almost a 90% reduction from the control group (Figures 6 & 7). Populations

remained below 0.5 log (less than 5 cells per gram of product) for the 20 day duration of the shelf life study. There were no significant differences between pressure/time treatments in *V. vulnificus*. This amount would not result in disease in most individuals, even many of those at risk.

Populations of *Vibrio parahaemolyticus* were reduced 2-3 logs (99-99.9 percent) by high pressure processing and remained lower than the control group for the study duration (Figures 8 & 9). The 275 MPa/30s treatment was less effective in *V. parahaemolyticus* during the fall, only yielding a 1.5 log reduction. There was no significant difference between the more effective 275 MPa/60s treatment and the 310 MPa/30s treatment in the fall. There were no differences between pressure/time treatments in the treatment of *V. parahaemolyticus* harvested in the summer.

The initial populations of *Vibrio* species investigated were similar in the fall and summer oysters, although a greater decrease in control populations was observed in the oysters harvested during the summer. The oysters harvested in the fall contained populations of *Vibrio* that were more resilient to storage conditions. One possible explanation for this effect is that summer water temperatures are similar to the optimal growth temperatures for *Vibrio*, between 30° and 35 °C. At these warmer temperatures the bacteria are less stressed and may be more susceptible to other stressors such as high pressure processing and low temperature. During the fall, when temperatures are cooler, the bacteria present are more stress resistant than their summer counterparts.

Physical properties

High pressure processing can reduce the need for manual shucking by releasing the abductor muscle from the shell. All treatments decreased the percent of unshucked oysters from 100% in the control to 10% or less (Table 1). More than 80% of the oysters in the HPP treatments were partially shucked. Processing pressures had a greater effect on the number of oysters that were fully shucked than processing time. Oysters that were partially or fully shucked received less damage during mechanical shucking which could allow for the use of less skilled labor needed and therefore lower labor costs.

Maintaining a low driploss in shucked oysters is essential from both a quality and regulatory standpoint. Legal requirements for driploss state that shucked oysters may not have a driploss greater than 10%. In this study, driploss was shown to increase with the use of high pressure processing treatments. Initial driploss was greater in the treatment of 275 MPa for 30 s although none of the treatments had a driploss of greater than 10%. Treatments all had a higher driploss than the control during the remainder of the shelf life study. Fewer differences were noted in the summer due to a higher variability that may be caused by bacterial degradation. The oysters from the summer study also had much higher driploss values than the fall study. Excessive driploss caused by high pressure processing may be attributed to either damage to the cell walls. Oysters may also imbibe water during processing, which is then lost over time.

High pressure processing did not affect the color of the oysters initially or over the shelf life of the study. High variability in the color of oysters may have caused no differences to be found in the color using instrumental analysis. Observations by a small informal panel noted that the oysters treated with HPP had a slightly whiter coloration than the control oysters. The whiter color could be an indication of some protein denaturation during the HPP treatment.

A sensory study measuring differences in odor showed no olfactory differences between treatments, including the control, and the fresh frozen oysters presented until 15 day of shelf life storage. At day 15 differences were noted in the control and 275 MPa / 30 s treatments and the fresh frozen oyster. After 20 days all treatments were identified as having a different odor than the control. This suggests that high pressure processing can extend the time for which oysters are considered having a "fresh" odor when HPP parameters of 275 MPa for 60 s and 310 MPa for 30 s are utilized.

CONCLUSIONS

The use of HPP in the oyster industry is currently being employed to both increase the safety of the product used for raw consumption and to eliminate the need for skilled labor during shucking. The use of high pressure processing oysters can be an effective non-thermal treatment for reducing both spoilage and pathogenic microorganisms in Eastern oysters. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are pathogens of public health significance in the raw shellfish industry that can be reduced using the HPP parameters used in this study. Bacterial decreases of this nature are an important step to increasing the safety of a product that is often transported to different locations before purchase and consumption.

Populations of fecal coliforms are also reduced with HPP and remained lower than the control during the length of the shelf life study. Results of sensory and aerobic plate count analysis also indicated that shelf life extension can be achieved with HPP technology. The physical characteristics of oysters tested in this study showed few differences with HPP treatment. The treatments showed no sensory or color differences from the control at the beginning of the study. Driploss is increased by the use of high pressure and may require further testing to determine if methods can be developed to reduce excessive driploss. Release of the oyster abductor muscle during HPP can be an added economic benefit to the technology.

Table 1. Shucking effects on oysters due to HPP

HPP Treatment	Release of abductor muscle after HPP treatment		
	None	Partial	Full
Control	100%	0%	0%
275 MPa / 30 s	10%	83%	7%
275 MPa / 60 s	0%	95%	5%
310 MPa / 30 s	0%	87%	13%

100 oysters were used for each treatment

Table 2. Driploss of oysters treated with HPP during refrigerated storage.

Season	Treatment	Storage time				
		2 d	5 d	10 d	15 d	20 d
Summer	Control	6.2%	13.0%	17.9%	17.8	20.3
	275MPa/30s	9.2%	15.2%	19.1%	24.8	20.2
	275MPa/60s	10.9%	13.2%	17.5%	21.9	23.0
	310MPa/30s	9.9%	15.2%	17.2%	24.6	23.6
Fall	Control	5.0%	5.52%	4.4%	6.5	9.6
	275MPa/30s	7.2%	7.5%	10.5%	9	13.1
	275MPa/60s	4.6%	9.4%	11.5%	7.4	15.1
	310MPa/30s	5%	9.2%	9.5%	7	13.3

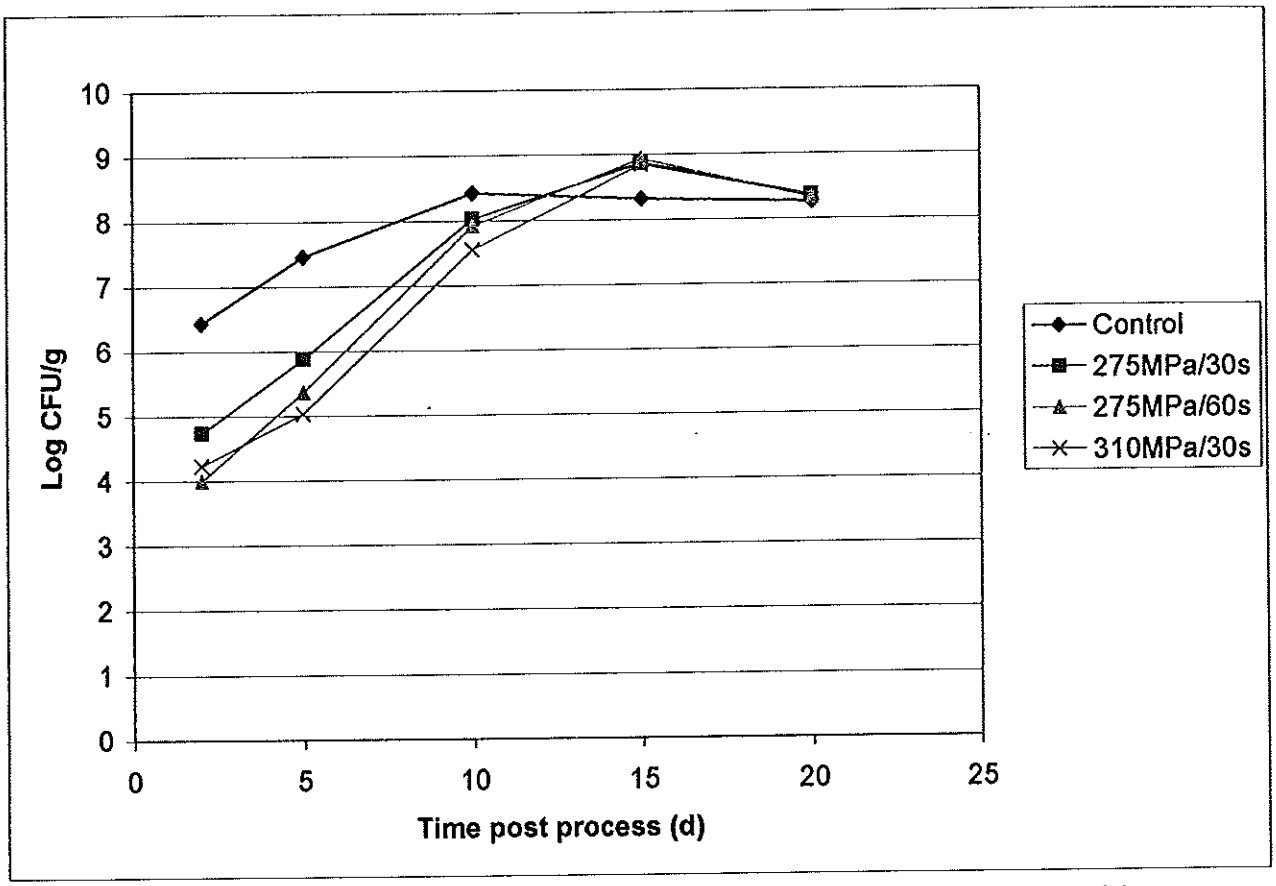

Figure 1. Aerobic plate count over the shelf life of fall harvested raw oysters treated with High Pressure Processing.

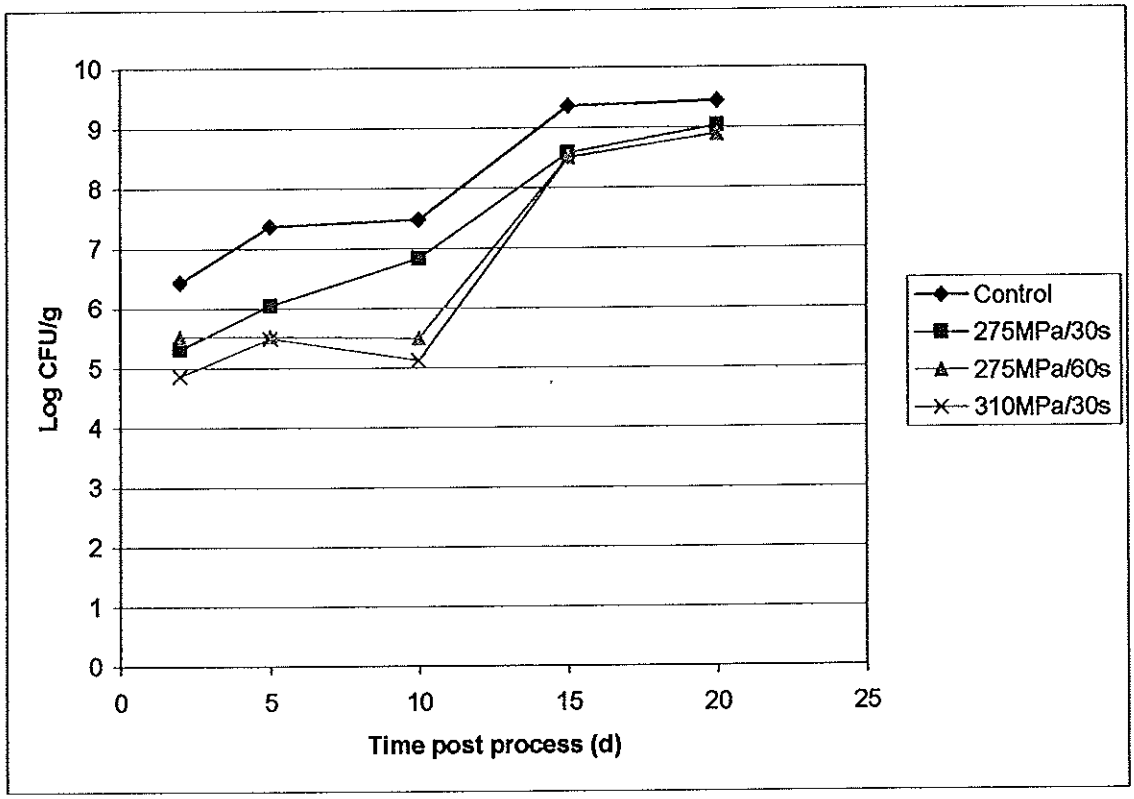

Figure 2. Aerobic plate count over the shelf life of summer harvested raw oysters treated with High Pressure Processing.

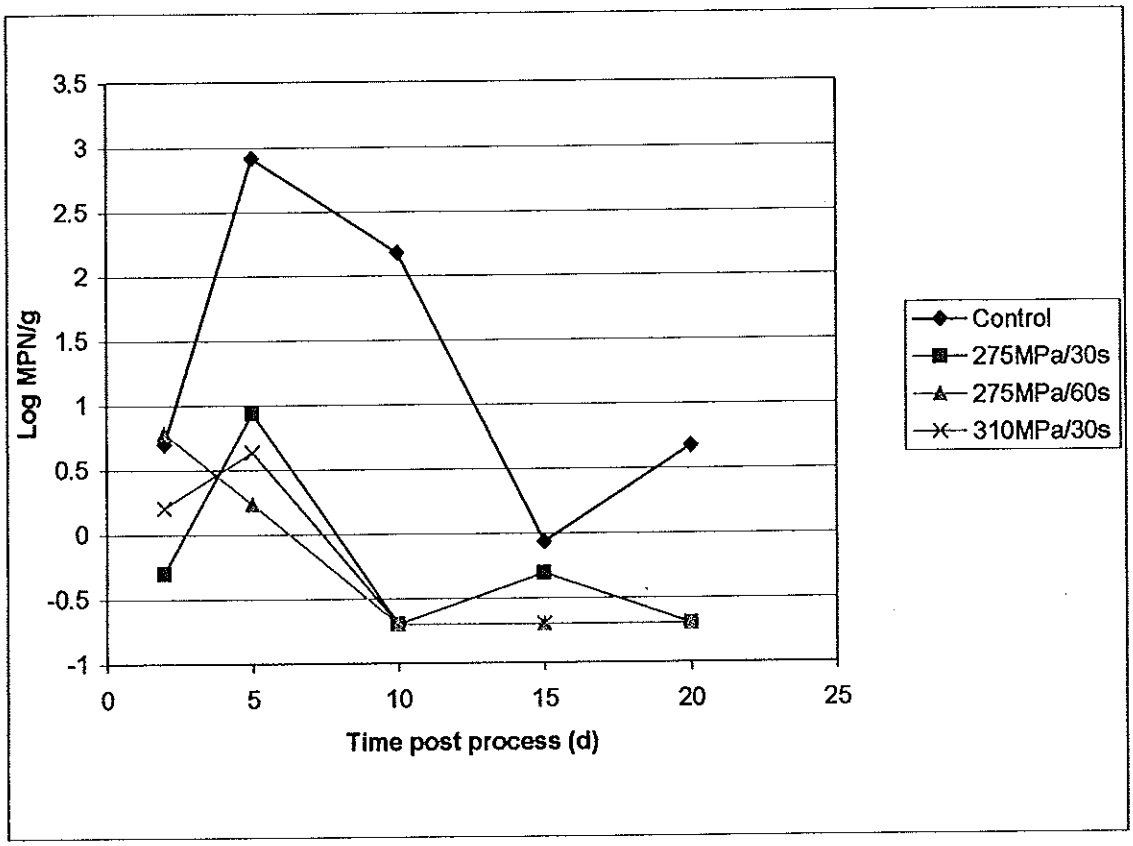

Figure 3. Population of fecal coliforms over the shelf life of fall harvested raw oysters treated with High Pressure Processing.

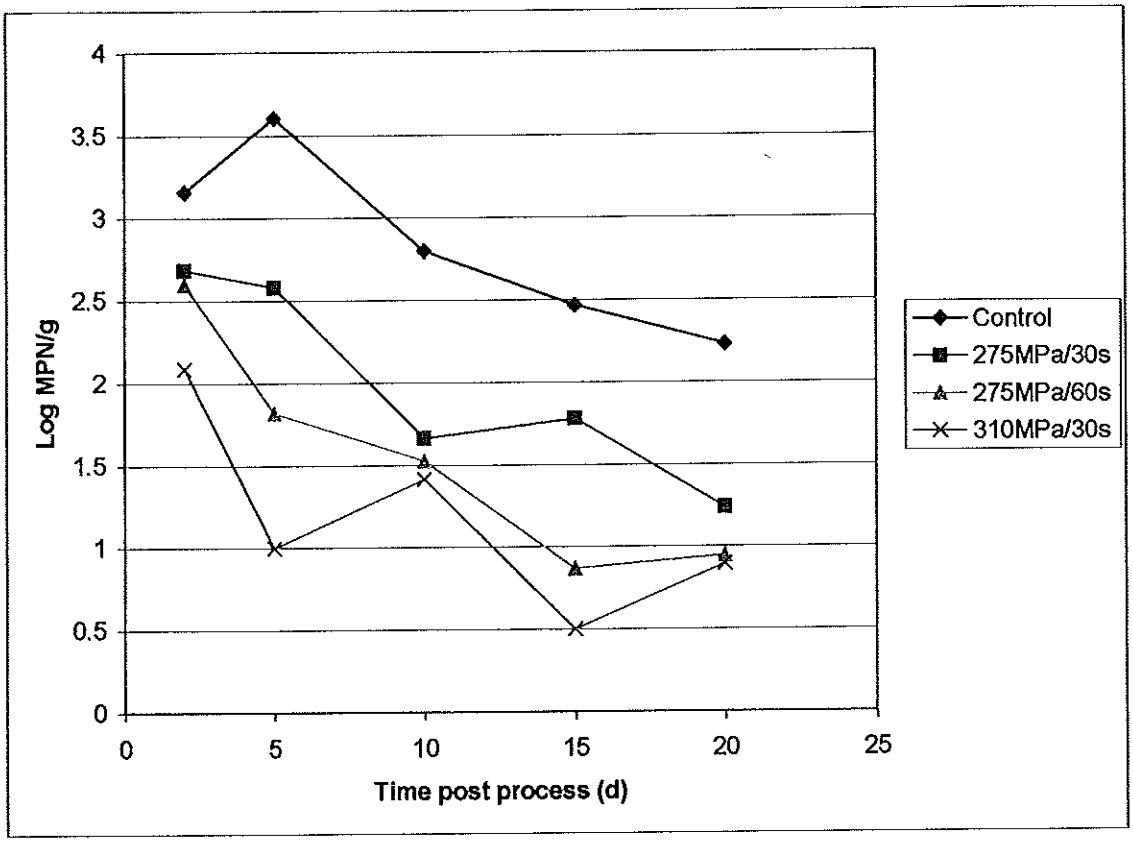

Figure 4. Population of fecal coliforms over the shelf life of summer harvested raw oysters treated with High Pressure Processing.

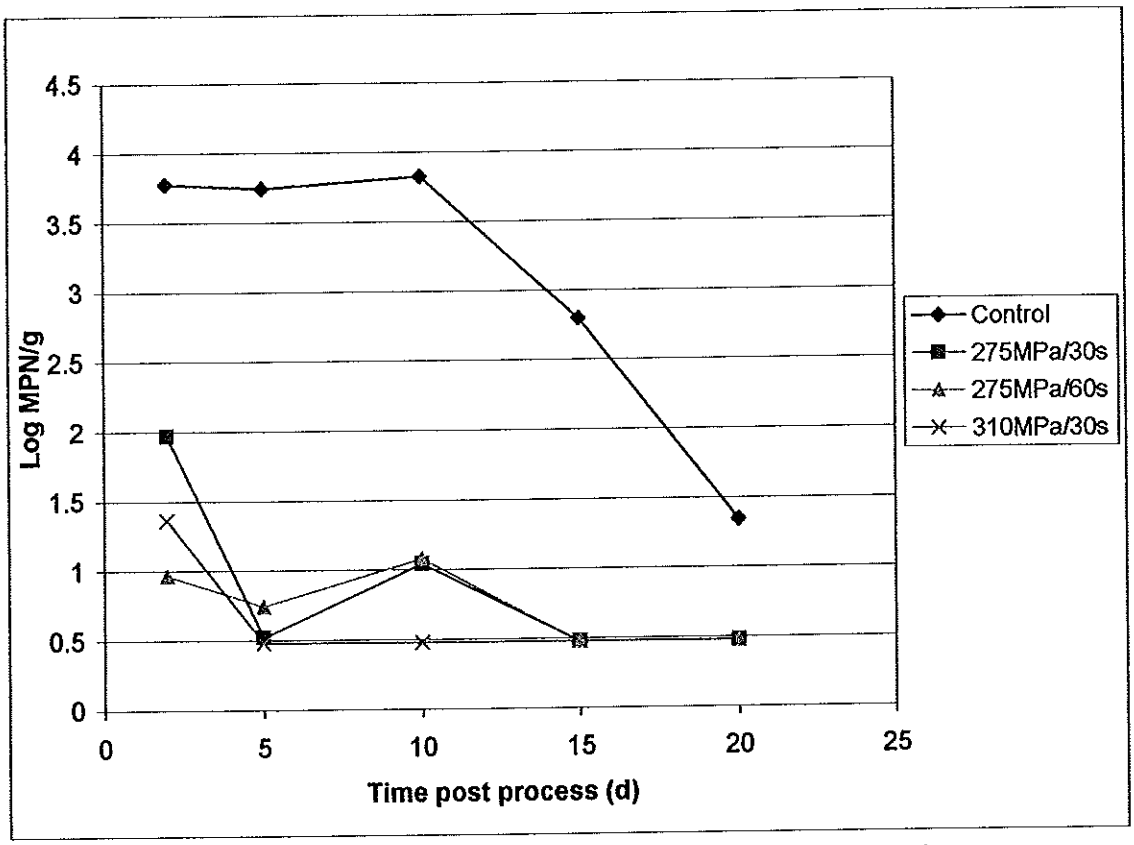

Figure 6. *Vibrio vulnificus* population over the shelf life of fall harvested raw oysters treated with High Pressure Processing.

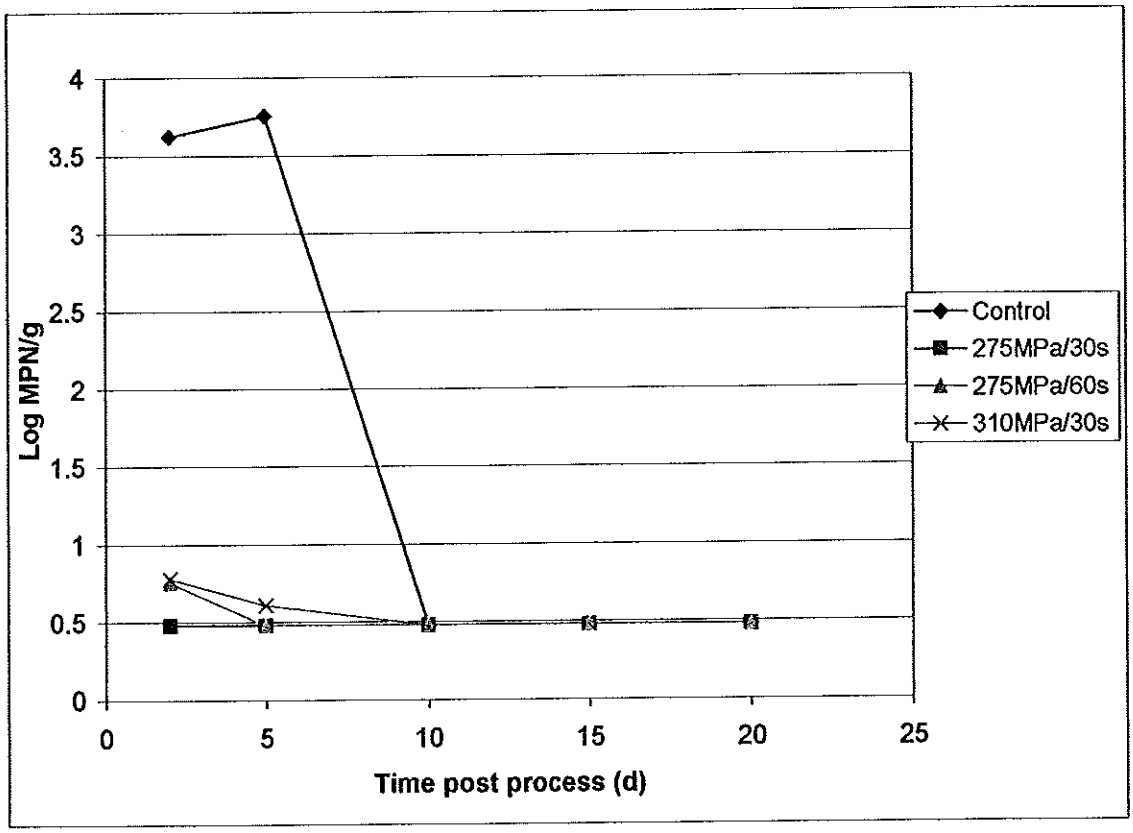

Figure 7. *Vibrio vulnificus* population over the shelf life of summer harvested raw oysters treated with High Pressure Processing.

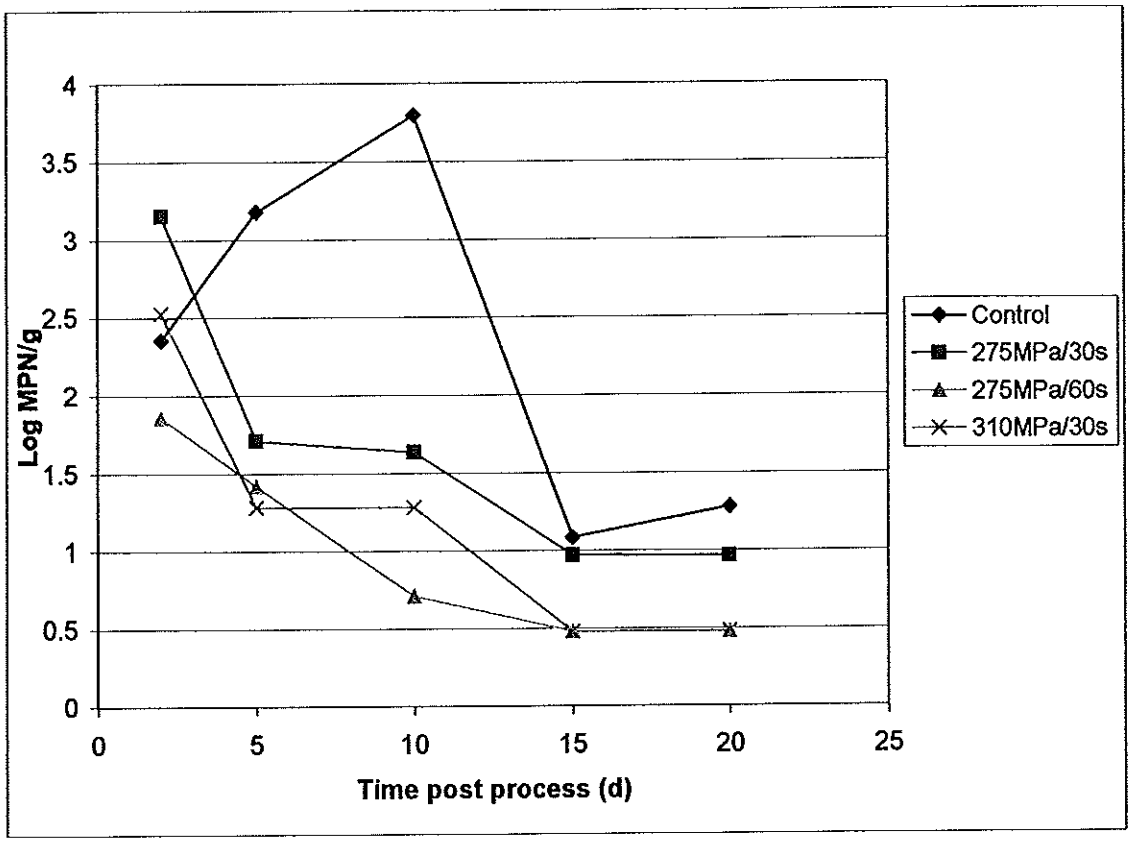

Figure 8. *Vibrio parahaemolyticus* population over the shelf life of fall harvested raw oysters treated with High Pressure Processing.

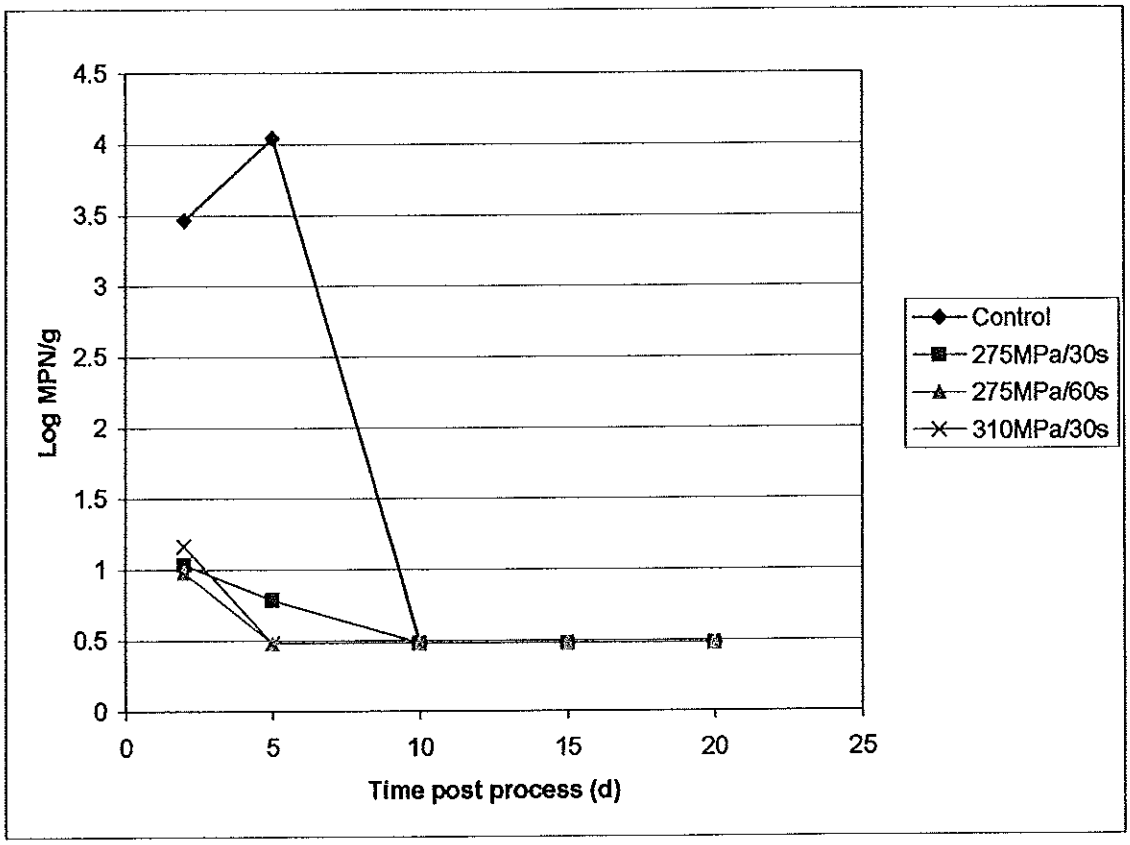

Figure 9. *Vibrio parahaemolyticus* population over the shelf life of summer harvested raw oysters treated with High Pressure Processing.

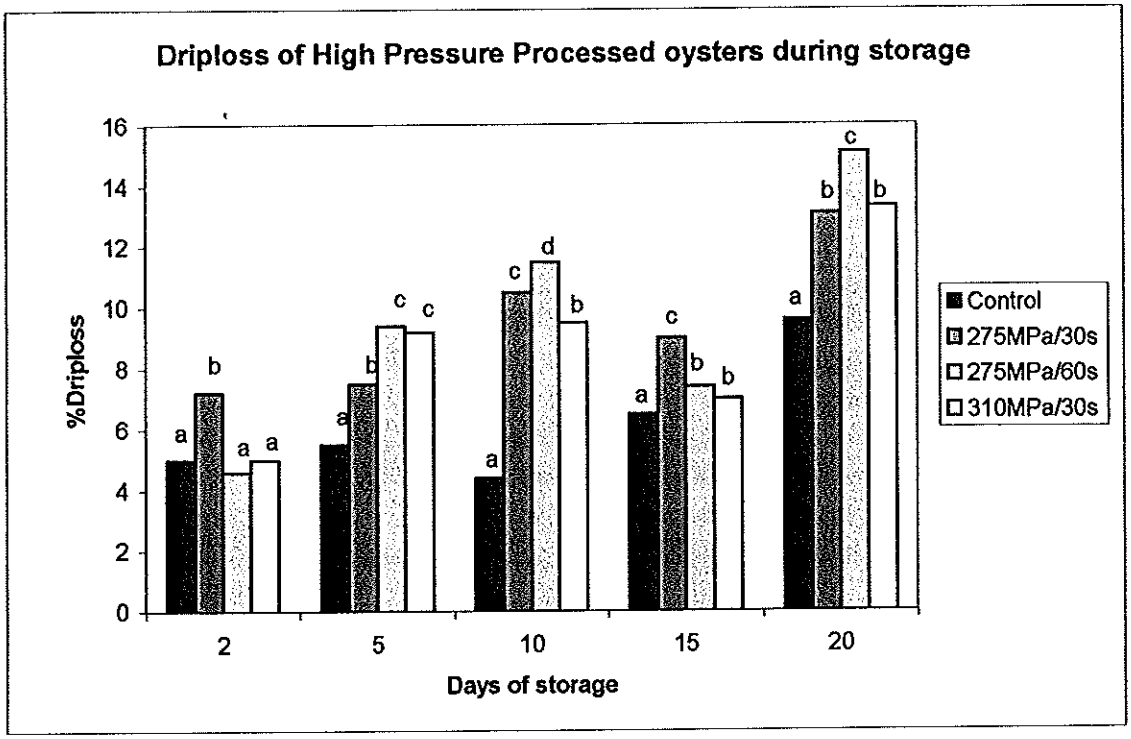

Figure 10. Percent driploss of HPP treated fall oysters during refrigerated storage (4 °C).

Chapter 2

PURPOSE

- To introduce high hydrostatic processed clams and oysters to retail and food service customers for evaluation.

- To demonstrate high hydrostatic processed oysters and clams at the International Boston Seafood Show.

- To arrange high hydrostatic pressure demonstrations on Western and Eastern shores of the Chesapeake Bay region of Virginia so that all shellfish dealers can have access to view and use the machine for product market evaluations.

RESULTS

High pressure processed clams and oysters were demonstrated in the Virginia Tech both at the 2004 International Boston Seafood Show. There was also additional seafood items displayed including crabmeat, surimi, cooked shrimp, oysters in cocktail sauce, and smoked salmon. The display challenged participants to find visual differences between the high pressure processed sample and a control. Participants commented that they were unable to identify a difference.

A high pressure processing workshop was presented by Virginia Tech in May of 2004. The Virginia oyster and the clam industry was invited to participate and the two day conference was presented free of charge to all attendees. Conference sessions included the fundamentals of high pressure processing, equipment description and operation, case studies of products successfully introduced into the market, packaging requirements, and effects of high pressure processes on bacterial, viruses, and parasites which were all relevant to the Virginia fish and shellfish industries. Sessions at the Virginia Tech Department of Food Science and Technology included a demonstration of HPP equipment, sensory panels of HPP foods currently available on the market, and a tour of other facilities available for testing within the department. Participants were also given the opportunity to shuck oysters, which were treated with HPP, and a set of control oysters. Gold Band Oysters, a brand of HPP treated oysters, were also presented at the evening reception for sensory evaluation.

The high pressure device could not be demonstrated in the coastal areas of the state. The device is 15 feet tall, weighs 20,000 pounds, and requires 55,000 watts of electricity delivered at 440 v. Because of the height requirement when placed on a low truck, over 17 feet, special transportation was required to move the device from the Norfolk port to Blacksburg and from the delivery vehicle into the Food Science and Technology Department. Moving the device to the coastal area would have been economically prohibitive.